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EFFECTS OF LITHIUM
ON
URINARY ACIDIFICATION

Robert A. Sirota

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EFFECTS OF LITHIUM ON URINARY ACIDIFICATION

by Robert A. Sirota

B. S. Long Island University 1969

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1973

to my parents

to Karen

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Literature Review: The Effects of Lithium on the Kidney

Since its isolation in the mineral petalite in 1817, salts of the element lithium have been utilized by the medical profession with varying degrees of enthusiasm in the treatments of diverse clinical disorders. Originally enjoying only sporadic popularity as a uric acid solvent in the treatment of gouty arthritis and rheumatism (145), its first widespread clinical application was as a taste substitute for sodium chloride in the treatment of fluid overload states (127). However, a series of reports in the early 1950's of deaths due to lithium overdose in these conditions (16,48, 50,84) ultimately put an end to this therapeutic use. In 1949 an observation made in Australia (10) suggesting that lithium might have a beneficial effect in the treatment of mania led to renewed interest in its pharmacotherapeutics. In the subsequent two decades lithium has been shown to be efficacious in the treatment of affective disorders (22,40, 46,76,108,110) and had enjoyed widespread popularity in this area. In accord with this extensive clinical usage, there has grown an impressive list of lithium-induced side effects (20,84,111,112) unrelated to its psychotropic effect. Certain of these effects have been of decided nephrologic interest, and are the impetus for the studies herein described. It

is the purpose of this introduction to review what is presently known of the renal and cellular effects of lithium, in an effort to interpret the present experiments.

I. Renal Excretion of Lithium

It has been known from the earliest experimental efforts (45,66) and reaffirmed in recent studies (63,88,99,128,132) that at least 95% of lithium ingested in both experimental animals and man is excreted by the renal route. Lithium remains unbound to plasma proteins (35,128), and is, hence freely filterable at the glomerulus. Normally in humans from one-third to two-thirds of a moderate (27-50 meq./70kg.) acute oral dose of either lithium carbonate or chloride is eliminated in the urine in the first day (30,88,132), with almost complete excretion within one (96) to two weeks (132). Human clearance rates have been estimated at about 15-30 ml./min. (111), corresponding to a C_{Li}/C_{Cr} ratio of about .2 (129,130,131). The latter figure was derived from a single seven hour clearance experiment in six healthy human volunteers without dietary restrictions who had taken an acute oral dose of lithium (16.2 meq.) ten hours before urinary collection. It represents the only documented value for human urinary clearance of lithium. Corroborative studies in the dog (35,99) and the rat (107) demonstrated clearance ratios of between .2 and .4, independent of

serum lithium concentrations for values greater than 1 meq./L. Below this value the latter study suggested that clearance ratios become inversely proportional to serum lithium concentrations, approaching unity as serum lithium concentration approaches zero.

Lithium clearance ratios in the rat have also been shown to vary with net sodium excretion (107), with higher sodium excretion causing higher lithium clearance. Additional evidence from human studies (130) suggests that lithium clearance increases when sodium intake is augmented over long periods of time. (Such an increase was not, however, able to be demonstrated with acute oral saline loading.) These phenomena may be a partial explanation for the findings of previous studies which suggest 1) that dogs and humans seem more susceptible to the toxic effects of lithium when sodium intake is restricted (16,48,50,99,125,127) and 2) that lithium treated rats fed low sodium diets demonstrate chronically higher plasma lithium levels than those fed high sodium diets (106).

An excretion fraction ranging between 20 and 40% of filtered lithium in the whole kidney is similar to the excretion fraction of sodium from the proximal tubule in both the rat (47,134,142) and the dog and man (138). This, coupled with the evidence suggesting that tubular rejection of lithium occurs with sodium loading, a phenomenon similar to the tubular

rejection of sodium which occurs with acute saline infusion (18,25), has prompted certain investigators (131) to hypothesize that lithium is resorbed proximally in a quantitatively similar fashion to sodium, with little distal tubular reabsorption. Such a conclusion is supported by human experiments (130) which indicate that fractional lithium resorption is unaffected by administration of thiazides, ethacrynic acid, and spironolactone in doses sufficient to produce a 2-3 fold increase in sodium excretion, (presumably by causing tubular rejection of sodium at various distal sites) (114), and by experiments (130) which indicate enhanced tubular rejection of lithium under conditions of urea or bicarbonate diuresis, both of which are known to diminish proximal tubular sodium reabsorption.

There is evidence in older dog clearance experiments (35), however, in which administration of mercurial diuretics (whose presumed site of action is in the proximal tubule (114)) induced no change in lithium clearance ratios, which casts some doubt on an exclusive proximal resorptive mechanism. Subsequent evidence from stop-flow experiments in dogs (62) has implicated some distal lithium resorption, possibly in exchange for potassium secretion (although previous work (35) demonstrated no effect of potassium loading on lithium excretion patterns). There has also been the observation from tissue

analyses experiments in dogs (123) and rats (34) that there is a 2-3 fold corticomedullary gradient for lithium established during periods of lithium loading, suggesting lithium resorption distal to the terminal end of the proximal tubule. Finally, there is a recent report of triameterine induced lithium diuresis in humans (4), which suggest that lithium may be handled at least in part at aldosterone-insensitive distal tubular resorptive site. Thus, while the weight of evidence favors a predominant proximal resorptive site for lithium, the existence of distal tubular lithium resorption has not been ruled out.

II. Renal Cellular Transport of Lithium

Because of variation in the clearance of lithium with sodium excretion (vide supra) it was postulated in the older literature that lithium may in some way compete with sodium for tubular resorption (107). Support for this hypothesis is found in studies of the relationship between lithium and sodium transport found in other cellular systems. Thus, it was demonstrated as early as 1902 (92) and reaffirmed in innumerable subsequent experiments (11,19,39,58,59,64) that lithium was unique in its ability to substitute for extracellular sodium in supporting both resting and action cell potentials. Lithium was subsequently shown to compete with

sodium for entry into red blood cells (56,122) and mucosal entry into toad bladder cells (36). Lithium is also capable of replacing sodium (and potassium) in stimulating various extracts of sodium-potassium ATPase (116,117,118,139,140,141), (an enzyme felt to be important in active transport of sodium across the peritubular membrane of the renal cell (65)) and to inhibit active sodium transport out of red cells, possibly through interaction with this enzyme (67). Lithium transport in toad bladder has also been shown to be partially ouabain sensitive, a characteristic shared with sodium ion in this preparation (57). Thus there is indirect evidence to suggest that lithium may be transported by the same mechanism which transports sodium at the cellular level. Such an assumption, however, concedes that lithium, like sodium, is actively transported at the cellular level. Active transport of lithium has been directly demonstrated in only one study (147), in which it was reported to be transported across a toad bladder preparation against an electrochemical gradient. In contradistinction to this data is more recent toad bladder data (57) suggesting that lithium is unable to cross an unfavorable energy barrier of 460 cal./eq. In addition, numerous studies from work in red cell (32,68,77,78) and kidney slice (17,69) systems suggest that lithium can neither be transported actively into or out of the cell. Lithium has, rather, been shown to move

passively through these cellular membranes, with a rate constant similar to the passive rate constants for sodium and potassium movement (55,77) (which is 10 to 25 time slower than the active efflux rate constant for sodium (67,68)),and to maintain unaltered its own efflux rate in both red (68) and kidney (69) cells when active sodium-potassium exchange was inhibited by potassium-free medium, or ouabain, respectively. Such data make it unlikely that lithium can be transported by a sodium transport system which relies on active pumping of the ion out of the cell.

In summary, then, present evidence indicates that lithium cannot be transported against an electrochemical gradient either out of the cell or across an epithelial membrane; however it does share other transport-related characteristics with sodium. From this position, it is difficult to imagine that lithium is transported in the tubule by a mechanism similar to that postulated for sodium, which, as presently conceived by some (93), relies on active transport of sodium from peritubular surface of the renal cell, with subsequent passive transfer of tubular sodium through the cell. One rather may favor the resoprtive mechanism proposed by Giebisch (41,42) wherein sodium is actively resorbed by a peritubular pump through both intracellular and extracellular channels (the tight junctions of the proximal tubule), with other ions

(including lithium) and water following passively through the latter. Such a proposition rids one of the necessity of explaining how lithium would be extruded from the renal cell once it enters it. Or one might be tempted to hypothesize that lithium is transported by the ouabain-insensitive, peritubular ion transport pump, proposed by Kleinzeller (69) to explain isosmotic shifts of ions (lithium included) and water in ouabain-inhibited kidney cortex cells. This latter possibility does not require pumping of cellular lithium against a peritubular gradient. Until more direct work is done on renal tubular transport of the lithium, it is impossible to do more than conjecture about these and other plausible mechanisms for renal lithium resorption.

III. Effects of Lithium on Renal Function

There remain to be discussed a number of nephrologic effects of lithium which may represent pharmacologic effects of the ion on tubular function rather than by-products of its own renal handling. It has been noted in many studies (3,63,87,132,133) that chronic lithium ingestion in humans causes altered patterns of urinary sodium excretion, with abnormal urinary sodium loss on the first day being followed by net lithium retention on subsequent days (the longest experiment was one week in duration). In an additional study (23)

nine out of nine patients with no predisposing causes developed pretibial edema shortly after lithium carbonate therapy was begun (although no net sodium retention could be documented by urinary sodium determinations). These patients became asymptomatic after lithium therapy was discontinued. Balance estimates in chronic dog (99) and rat (106) experiments have failed to show similar retentive patterns, and, in fact, indicated chronic urinary sodium loss shortly after parenteral lithium administration was begun. However, both dogs and rats at this time were already showing symptoms of renal toxicity (see below) and results, therefore, are not comparable to human studies.

Mechanisms for sodium retention during lithium therapy have not been well elucidated. Extrarenal changes in total sodium distribution and total body water have been both documented (2,14) and disputed (15) during lithium treatment, and while results are contradictory, it is possible that lithium-induced changes in these parameters form the basis for observed aberrations in urinary sodium excretion. It is likewise plausible that lithium may be causing sodium retention through an adrenocorticoid mediated mechanism. A decrease in pretibial edema in the nine patients previously mentioned when placed on spironolactone (23) suggested this possibility to the authors of this study (even though urinary aldosterone excretion was

normal). More directly, Platman et al (97) found abnormally elevated serum cortisol levels in 8 out of 11 patients placed on moderate doses or oral lithium carbonate five days before study and conjectured that lithium may in some way be facilitating cortisol secretion. More recent evidence from a similar study (104), however, demonstrated normal plasma cortisol and cortisol production rates in ten patients on chronic lithium therapy. Contradictory results, therefore, again make positive interpretation of data impossible. A third explanation for abnormal sodium excretory patterns after lithium may lie in a direct effect of lithium on the renal tubular cell, but evidence in support of such a hypothesis is lacking.

Lithium has also been found to alter potassium metabolism. Net urinary potassium loss has been demonstrated the first day after lithium ingestion in humans (3,132). A similar finding has been demonstrated only minutes after intravenous administration of lithium chloride in anesthetized dogs (7,35). This study also demonstrated that when potassium excretion was raised to maximal levels by potassium loading before lithium infusion, no further rise could be elicited with subsequent lithium infusions, suggesting to the investigators that its action was occuring at a distal secretory site; mercurial diuretics were also able to reduce the potassium response to lithium. This series of experiments also demonstrated

that potassium loading did not alter lithium clearance values (see above). It is, therefore, unlikely that the mechanism of lithium's action resides in tubular resorption of the ion in exchange for potassium at a distal site. Rather, lithium's action here may be related to a cellular exchange of a certain fraction of tubular lithium and intracellular potassium, with gross net lithium resorption not being affected.

Another area of considerable nephrologic interest has concerned the action of lithium on urinary concentrating mechanisms. A diabetes insipidus-like syndrome in patients taking lithium salts was first noted in 1954 (132); subsequently, reports of polyuria and polydipsia as a side effect of lithium therapy have appeared sporadically in the psychiatric literature (20,40,105,108,109), but it was not until recently that the entity has become well documented in careful clinical investigation (1,33,34,74,120). Originally felt to be a rare complication of lithium therapy, Forrest et al (34), in a study of 96 consecutive patients treated at a large lithium clinic, have documented symptomatic polydipsia in 40% and polyuria (greater than 3000 ml./day) in 12%, at a time when serum lithium concentrations were normal. When ten patients were prospectively studied before and five weeks after lithium therapy was begun, 70% showed significant decreases in maximum urinary concentrating ability (as judged by a dehydration plus

vasopressin regimen proposed by Miller (85)). Thus, rather than rare, lithium-induced concentrating defects stand as a most prominent side effect of lithium therapy.

Even more striking effects on renal concentrating mechanism have been found in animals. Large increases in urine volume were first noted after chronic lithium ingestion in dogs in 1950 (99). A 1958 study by Schou (106) documented the rapid development of marked polyuria (up to 400 ml./kg./day) in 100% of rats receiving daily intermediate intraperitoneal doses of lithium chloride (3-7 meq./kg. rat body weight/day). Similarly, Forrest et al (34) have documented significant increases in rat urinary volumes only one day after 3-4 meq./ kg. rat body weight/day doses of intraperitoneal lithium chloride was begun, with daily urine output to nearly 50-70% of total body weight within four to five days. No similar increases were noted in sodium chloride injected controls. Finally, Harris and Jenner (52,54), working with rat in vivo vasopressin assays, found a significant drop in vasopressin sensitivity shortly after hypotonic lithium chloride administration was begun, and documented similar levels of inhibition at widely varying serum and total body lithium concentrations (53).

There have been many recent attempts to elucidate mechanisms for lithium induced polyuria. It has been found in human studies (1,33,34,74,120), whole animal vasopressin concen-

trating tests (33,34,106) and in vivo rat vasopressin assays (52,54) that lithium induced concentrating defects are unresponsive to exogenous vasopressin administration (although two human studies (34,120) have shown submaximal vasopressin responses in rare polyuria patients). That increased thirst is not a major factor in the effect (as was suggested by Smith et al (121)), was successfully demonstrated by Forrest et al (34) who reported differences in vasopressin-stimulated concentrating ability between lithium-polyuria rats and similarly polyuria glucose-water drinking controls. Further study in humans (120) demonstrated normal free water clearance (thought to be indicative of intact function of the distal loop of Henle and early distal diluting segment) in these lithium polyuria patients. In addition, rat (34) and dog (123) studies have shown normal corticopapillary gradients for sodium during lithium polyuria. Together these findings suggest intact function of the countercurrent mechanism for generating medullary hypertonicity in lithium-induced polyuria. Thus, the remaining plausible explanation for the lithium-induced defect in urinary concentration lies in vasopressin insensitivity at the distal nephron level. Forrest et al (34) has been able to show inhibition of urinary concentration after both vasopressin and dibutyryl cyclic AMP infusion into lithium rats, supporting such a hypothesis and suggest that lithium's action may be in-

hibitory at steps beyond cyclic AMP mediated distal tubular phenomena.

There are pertinent toad bladder studies which both support and refute such a hypothesis. Comparable to Forrest's *in vivo* finding, Harris and Jenner (54) reported inhibition of vasopressin and cyclic AMP stimulated water flow in toad urinary bladder at low serosal concentrations of lithium (2 meq./L.). In contrast, although Singer (120) demonstrated inhibition of vasopressin-stimulated increases in both short circuit current and osmotic water flow at moderate lithium concentrations (11 meq./L.), lithium was only active in this way when placed on the mucosal side and, furthermore, the cyclic AMP stimulated increases in short circuit current and water flow were *unaffected* by the presence of lithium. Conflicting with both results is a report by Bentley and Wasserman (5), who, although demonstrating inhibition of both basal vasopressin and cyclic AMP stimulated short circuit current with moderate lithium concentration, found this effect with *both* mucosal and serosal lithium; furthermore, these authors *failed* to demonstrate any lithium-induced inhibition of vasopressin-facilitated water flow with even higher concentrations of lithium (40 meq./L.) placed on either side of the membrane. Although these inconsistencies in toad bladder studies are not resolved, all data delineate some effect of

lithium on vasopressin-mediated toad bladder transport phenomena, and, in this way support the conclusion that lithium's polyuric effect in vivo is caused by altered response to vasopressin at the distal nephron level.

In relation to lithium's effect on the adenyl cyclase-cyclic AMP system, it should also be noted that this ion has recently been found in in vitro assay to inhibit vasopressin-stimulated adenyl cyclase isolated from rabbit (29) and human (27) kidney medullary extracts, parathyroid stimulated adenyl cyclase extracts from beef thyroid gland (144), and adenyl cyclase extracts from brain (28) and fat cells (8), thus lending additional support to evidence placing its action at this biological site in the kidney.

As to therapy of lithium-induced polyuria in humans, the only reported results (34) found vasopressin tannate in oil and chlorpropamide to be of no therapeutic benefit, while chlorthiazide was successful in reducing urinary volume. A report demonstrating abolition of lithium-induced vasopressin unresponsiveness in toad bladders through mucosal acidification (to pH 5.1) (119) prompted a trial of ammonium chloride loading in two patients (34) but results of this attempt to control lithium polyuria through urinary acidification was inconclusive.

Aside from renal concentrating defects and electrolyte excretion abnormalities, nephrotoxicity with termal renal fail-

ure and azotemia has been ascribed to a toxic action of lithium in scattered reports of fatal (16,112) or near fatal (49,125) lithium poisoning in humans; in the latter case renal biopsy suggested a direct nephrotoxic effect of lithium to the authors. However, the terminal illnesses of these patients make it impossible to ascribe end stage azotemia to a direct effect of high serum lithium levels. In this regard animal studies are more relevant. Radomski (99) demonstrated gross sodium wasting and preterminal oliguria in previously polyuric dogs kept on chronic daily oral lithium carbonate administration for 12 to 30 days, and found renal distal tubular disorganization and necrosis at autopsy. Similarly Schou (106) found chronic sodium loss and preterminal oliguria and azotemia in previously polyuric rats at different intervals (which varied with daily sodium and lithium intake) after inception of chronic daily intraperitoneal lithium administration. In this study, severe proximal tubular degeneration and cellular damage occurred. Most recently, Evan (31), although not mentioning oliguria or salt loss, found in electron microscopic examination an increasing amount of renal tubular cell damage (primarily distal) as dogs were kept on higher doses of both lithium chloride and lithium carbonate for increasing periods of time. These data thus suggest a direct nephrotoxic effect of high doses of lithium, with both salt wasting and deteriorating renal func-

tion appearing after long exposure to high serum lithium levels. It is impossible, however, to ascribe terminal renal failure and pathologic changes to a direct effect of lithium on the kidney, as other factors such as gross fluid and electrolyte imbalance were occurring in the terminal stages of these experiments.

A last lithium-related nephrologic effect which has been reported sporadically concerns urinary acidification. In the only human study, Trautner et al (88,132) demonstrated urinary pH elevations of one to two units one to three hours after oral lithium chloride or carbonate ingestion. Good (45) mentioned a similar phenomenon in cats, while Orloff et al (91) and Berliner et al (7), in short communications reported acute elevations of urinary pH and urinary bicarbonate excretion, with simultaneous lowering of urinary titratable acidity after isotonic lithium chloride infusions in dogs. Accordingly Homer and Solomon (62), on the basis of stop flow experiments in two dogs infused with isotonic lithium chloride, concluded that lithium blocked normal distal urinary acidification. The quantitative aspects and the mechanism underlying the effect of lithium on acid excretion have not, as yet, been well defined. It is the purpose of the experiments presented below to elucidate further the quantitative and mechanistic aspects for the inhibitory role of lithium in acid excretion.

Experimental Method

I. Rat Studies

A. Acid excretion studies

Animals utilized in all the experiments were male Sprague-Dawley rats weighing between 140 and 180 grams. Rats were caged in groups of three until the beginning of an experiment and allowed Purina rat chow and water ad libitum up until that time unless otherwise specified. At the inception of an experiment, rats (usually 12 in an experimental run) were tared and induced by mild noxious stimuli to the tail to urinate as completely as possible into a test tube; this urine was saved for later pH determinations. They were then given an intraperitoneal injection of the appropriate ionic solution (either .15 M. LiCl, .15 M. NaCl, or .15 M. KCl) at a dose rate of 4 meq./kg. body weight and returned to their cages. One half hour later, they were again induced to void as completely as possible into another test tube (urine was saved for pH determinations) and then immediately given .5 M. NH_4Cl intragastrically in a dose of either 3.75 mM./kg. or 12.5 mM./kg. body weight. This was easily accomplished by one person by per orum gastric intubation with a number 6 French rubber catheter, while the rat was manually restrained. Following this procedure, rats were immediately placed into well cleaned in-

dividual metabolic cages in which they were allowed free access to water only. Cages were equipped to collect all voided urine under mineral oil; fecal contamination was prevented through the use of a fine wire screen placed between the rat and the oil. 140 minutes after introduction into the cages, the rats were induced to void for a third time into clean test tubes (for subsequent pH determinations) and were lightly anesthetized with an intraperitoneal injection of Inactin at a dose of 100 mg./kg. rat body weight. 150 minutes after introduction into the cages, the abdominal aorta was surgically exposed and arterial blood was collected into heparinized glass syringes. One half of this sample (approximately 2 cc.) was transferred anaerobically to a test tube partially filled with mineral oil, for subsequent plasma total CO_2 and lithium determinations, while the other half was left in the stoppered glass syringe, for subsequent arterial blood gas and pH determinations. Immediately thereafter any residual urine remaining in the bladder was drawn anaerobically into a tuberculin syringe and added to the urine collected in the metabolic cages. Urine collected at 140 minutes was also added to this amount after its pH was determined. The total collection was measured for volume; a portion saved under oil for subsequent total CO_2, electrolyte, and lithium determinations, while the remainder was diluted 1:25 with water for subsequent titratable

acidity and ammonia determinations.

Because of instability of all urinary acid parameters regardless of method of preservation (89), urinary pH determinations were performed within 30 minutes of urine collection. Titratable acid and ammonia determination were commenced within one hour of the completion of the final urine collection. In addition, because of slow but definite loss of CO_2 into the oil phase of the anaerobically collected urine and plasma samples, total CO_2 determinations were performed within four hours of collection.

B. Hematocrit study

18 male Sprague-Dawley rats between 140 and 160 grams were fed Purina rat chow and water ad libitum. At the beginning of the experiment, animals were randomly divided into three groups and tail vein blood was collected into heparinized microhematocrit tubes. Rats were then given intraperitoneal injections of either .15 M. LiCl, .15 M. NaCl, or .15 M. KCl at a dose rate of 4 meq./kg. body weight. 30 minutes later .5 M. NH_4Cl, in a dose of 3.75 mM./kg. body weight, was given intragastrically. 60 minutes later an additional sample of tail vein blood was collected into hematocrit tubes and rats were exsanguinated via abdominal aorta.

C. Electrolyte balance study

18 Sprague-Dawley male rats between 140 and 160 grams were randomly divided into two groups, paired for weight, and placed into individual metabolic cages, equipped for urine collection. Free access to water was allowed. The experimental group was allowed up to 12 grams per day of laboratory prepared rat food (containing per kg. diet 470 g. sugar, 300 g. casein, 100 g. lard, 50 g. corn oil, 6.1 g. NaCl, 11.6 g. K_2HPO_4, 50 g. vitamin-mineral mix) while each pair mate was allowed the same amount of food on the subsequent day. After two days of pair feeding, experimental rats were started on daily intraperitoneal doses of .15 M. LiCl at a dose rate of 4 meq./kg. rat bosy weight. Control rats received no injection. Rats were weighed daily and dosages were adjusted accordingly. The first urinary collection period extended for the 2½ hours after the first lithium dose, at which time urine funnels were emptied; the second urine collection period lasted 21⅓ hours; all subsequent urinary collection periods lasted 24 hours. Urine volumes were measured daily and aliquots of urine were frozen for subsequent electrolyte and lithium determinations. The experiment was terminated after the fifth 24 hour urine collection was completed, at a time when mean daily urinary volume was still rising.

II. Human Studies

Four healthy male volunteers and one psychiatric male in-patient judged to be manic depressive were involved in this pilot study of the effects of lithium administration on acid excretion in the human. Acid excretion was evaluated in each patient before lithium treatment and following a single oral dose of lithium, using a short acid excretion test described by Wrong and Davies (146). Subjects were allowed free access to food and water before and during the test. On the morning of the pre-lithium test, subjects emptied their bladders completely. Thenceforth, urine was collected hourly with a small amount of thymol as a preservative, and measured for volume and pH; a small aliquot was immediately frozen for subsequent titratable acidity and ammonia determinations. After the first baseline urine collection, a single tube of venous blood was drawn for serum total CO_2 determination. Subjects then swallowed a pre-determined number of 250 mg. uncoated NH_4Cl tablets (Lilly) over a 15-20 minute period at a dose rate of .1 g./kg. body weight. One additional venous blood was obtained for serum total CO_2 determination two hours after the NH_4Cl ingestion and the test was terminated three hours later.

On the day of the post-lithium test, a similar protocol was followed except that two hours before the NH_4Cl dose was administered, the subject ingested Li^+ at a dose rate of

28 meq./70 kg. body weight. In three cases this dose was in the form of uncoated lithium carbonate tablets while in two cases the dose was in the form of uncoated lithium chloride tablets. Again, blood was drawn at zero and two hours after ammonium chloride ingestion, this time for serum lithium as well as for CO_2 determination.

Determinations

Urinary volume determinations were made with volumetric urine collection test tubes with an accuracy of ±.1 ml. Urinary pH determinations were made using a Leeds-Northrup pH meter with an external calomel reference electrode and a glass reading electrode which read pH accurately in samples as small as .2 cc. All determinations were done in duplicate, with variance of less than ±.2 pH units.

Titratable acidity was determined by diluting between .2 and .5 cc. of urine in 25 cc. of distilled water, adding .5 cc. of phenolsulfonthalein (6 mg./ml.) as an indicator and titrating with approximately .01 N. NaOH to pH 7.4 (115). The titrating solution was standardized at each run with prestandardized .01 N. HCl. Titrations were done in duplicate, to an accuracy of ±.01 ml. and results were expressed as meq./ kg. rat/collection time by multiplying resultant concentrations by urinary volume/collection time/kg. rat.

Urinary ammonia was determined using a standard Conway microdiffusion technique (13) in which ammonia was released with saturated K_2CO_3 from 1 ml. of 1:25 dilution of urine placed in the outer rim of a sealed Conway dish. The resultant free ammonia diffused into 1% boric acid-Conway indicator placed in the center of the dish. This was back-titrated with prestandardized .02 N. HCl. Determinations were done in duplicate to an accuracy of ±.01 ml., and results were expressed as meq./kg. rat/collection time by multiplying resultant concentration times urinary volume/collection time/kg. rat.

Urinary and plasma total CO_2 values were obtained from Van Slyke gasometric analysis (136) on .1 cc. samples taken from under oil. Determinations were done in duplicate in most instances, to an accuracy of ±.5 meq., and results were expressed as meq./L. Serum bicarbonate concentration was determined by subtracting millimolar concentrations of pCO_2 determined on arterial blood gas samples.

In human experiments, serum total CO_2 values were determined by the Yale-New Haven Hospital Clinical Chemistry Laboratories.

Total acid excretion was calculated as excretion of titratable acid plus ammonia minus bicarbonate. Urinary bicarbonates were run only if urinary pH was greater than 6.25, as negligible values for bicarbonate excretion would be predicted

by the Henderson Hasselbach equation, using a U_{pCO_2} of 40, for urines whose pH was less than this amount (pCO_2 values exceeding this amount have not been demonstrated in urines within this acid pH range (26)).

Arterial blood pH and pCO_2 were determined by the Yale-New Haven Hospital blood gas laboratory, using an Astrup Radiometer fitted with a micro-glass pH electrode and a silicon membrane pCO_2 electrode.

Urinary and serum sodium and potassium values were determined in duplicate in appropriately diluted samples using flame photometry with an internal lithium standard. Urinary and serum lithium values were determined in duplicate using atomic absorption spectrophotometry.

Tail vein bloods collected in heparinized hematocrit tubes were spun at 3,000 r.p.m./min. for 3 minutes. Hematocrits were read to the nearest .5 per cent.

Statistical significance in the rat experiments was assessed by a non-paired Student's t test; in human experiments a paired Student's t test was used. Statistical confidence was established at the p .05 confidence limit. All results are expressed as means ± standard error of the mean.

Results

I. Rat Studies

A. Acid excretion studies

The acute effect of lithium chloride injection upon the rat's ability to excrete acid was compared to that of sodium chloride and potassium chloride in the first animal experiments described above. Two dosage levels of ammonium chloride were utilized, 3.75 mM./kg and 12.75 mM./kg., to induce both moderate and severe metabolic acidosis respectively (potassium chloride injected animals were studied during moderate acidosis only). The time course, urinary pH, and related data for the moderate acidosis experiments are shown in figure 1 and table 1. It is evident that although the three groups of animals are all excreting urine of similar pH before ionic injection (pH 6.6), the urinary pH of the lithium group dramatically diverges from that of the other two groups one half hour after the injections are given, changing from 6.59±.14 to 7.99±1.49, while urinary pH values in the other two groups are not significantly altered. That this response is not due to lithium induced metabolic alkalosis was shown in a separate experiment in which arterial blood pH values were found to be normal (mean = 7.36±.03) in a group of six rats 30 minutes after intraperitoneal lithium injection at a dose

of 4 meq./kg. Moreover, 150 minutes following this point, during which moderate metabolic acidosis was developing secondary to NH_4Cl ingestion in all the groups, the lithium group, although demonstrating some relative urinary acidification, failed to lower its urinary pH below that of arterial blood (urinary pH = 7.32±1.2, while arterial blood pH = 7.21±.02). At this point, both NaCl and KCl injected animals showed marked responses to the ingested acid load, with urinary pH of the former falling to 5.80±.05 and the latter to 5.97±.07. The difference in these two values was significant at the .05 level.

That true metabolic acidosis had been achieved in all groups at 180 minutes can be seen from the plasma HCO_3^- and arterial pH data of table 1, where it is noted that the lithium group exhibited the most severe acidosis, the sodium group exhibited the least severe acidosis, and the potassium fell in between the two. Differences in levels of acidosis among the groups also serve to elucidate the relationship between urinary responses of each group to ongoing acidosis. Thus, in a plot of urinary pH at 180 minutes vs. plasma bicarbonate (figure 2) it becomes clear that while the sodium group was responding to a slightly decreased plasma bicarbonate with an acid urine, the lithium group was responding to even lower levels of plasma bicarbonate with an inappropriately alkaline urine. Again in this figure, potassium chloride had an intermediary effect,

resulting in an acid urine at bicarbonate levels in between that of lithium and sodium.

Examination of other urinary parameters is in direct accord with the above results (table 1). Thus in the lithium group both titratable acid and ammonia are markedly decreased when compared to the other rwo groups, even though it is exhibiting the most severe metabolic acidosis; the sodium group demonstrated significantly greater excretion of titratable acidity and ammonia, while the potassium group, though excreting far more ammonia and titratable acidity then the lithium group, was unable to excrete ammonia as well as the sodium group (p .02). When total acid excretion, taken as the sum of titratable acid and ammonia minus bicarbonate excretion is studied, it is seen that the lithium group is least able to excrete the acid load, the potassium group assumes an intermediary potsition, and the sodium group demonstrates the greatest ability to excrete the acid load.

An additional finding which must be noted in the data of table 1 is the striking elevation of urinary volume in the potassium injected group when compared to the other two groups; V/2½ hr./kg. of the potassium group was 2½-3 times higher than that of the lithium or sodium groups, which demonstrated comparable values. Data for the potassium group represent the means from three separate experiments, all of which demon-

strated increased urinary volume

Having demonstrated an impressive defect in the excretion of a moderate acid load in lithium versus sodium or potassium injected rats, it was felt desirable to study acid excretion under more severely acidotic conditions in an effort to observe whether marked lowering of filtered bicarbonate will accentuate the inhibitory effect of lithium on acid excretion. In these experiments only sodium injected animals were used as controls. The time course and urinary pH data for these experiments appears in figure 3, while a summary of the remaining relevant data appears in table 2. The marked increase in urinary pH one half hour after lithium injection is again evident, but in contrast to the moderate acidosis induced 150 minutes after 3.75 mM./kg. intragastric NH_4Cl, 150 minutes after 12.5 mM./kg. of intragastric NH_4Cl a severe acidosis was induced, with plasma bicarbonate values clustering at about 8.5 meq./L. and arterial blood pH clustering at about 7.04 for both groups, with no statistical significance between them. In contrast to moderately acidotic conditions, severe acidosis produced a marked decrease in urinary pH in the lithium group from pre-acid conditions (from $8.23 \pm .10$ to $6.16 \pm .17$); likewise, sodium injected animals demonstrated a brisk decrease in urinary pH (from $6.65 \pm .062$ to $5.64 \pm .06$). Although statistical difference

between the two groups was achieved, it is apparent from figure 4 that in the very low plasma bicarbonate ranges, a few lithium animals closely approximate the minimal urinary pH exhibited in the sodium group. When excretion of titratable acidity, ammonia, and total acidity are studied, however (table 2), it is seen that there is still a large deficit in excretion of the acid load in the lithium injected group.

It is apparent from the above data that while lithium injection effectively blocks acid excretion, this inhibition is not absolute. Thus figure 5 illustrates that, similar to sodium injected animals, total acid excretion of the lithium injected group is significantly higher in severely acid animals than in moderately acidotic ones. This reflects significant enhancement of titratable acidity and ammonia excretion in both groups when more severe acidosis is imposed. Thus, the lithium-injected group, while impaired in acid excretion at all levels of acidosis, is able, like the sodium group, to vary its response appropriately with varying acid-base conditions.

B. Hematocrit study

This study was carried out to assess degrees of volume expansion in the sodium, potassium, and lithium injected animals

during the moderate acidosis experiments. As described in Experimental Methods, hematocrits were compared in rats at the beginning and 90 minutes after experimental inception; results are illustrated in figure 6. It can be seen that, although mean baseline hematocrits for each group were similar, 90 minutes later the lithium group demonstrated less hematocrit drop than either the sodium or potassium groups, which demonstrated similar depressions. The lithium group appears, therefore, to be the least volume expanded of the three groups, with both the sodium and potassium groups being equally more volume expanded.

C. Electrolyte balance studies

In an effort to relate acid excretory defects in lithium treated rats to other secretory or resorptive tubular defects, patterns of electrolyte excretion were studied both in the acute 2½ hour moderate acid loading model, and in a chronic rat balance study. In the former, two sets of preconditions were employed: in certain experimental runs, rats were allowed ad libitum Purina rat chow and water until the inception of the experiment, while in the others, rats were grouped carefully for weight and allowed Purina rat chow at a rate of 12 g./150 g. rat plus water ad libitum for 3 days before experimental inception. Thus the former groups of rats had

varying electrolyte intakes, while the latter had identical electrolyte intakes. Results for lithium and sodium injected groups appear in table 3, where it can be seen that in the electrolyte unbalanced studies there is no statistical difference in sodium or potassium excretion between the sodium and lithium injected group of rats. In the balanced experiments, both sodium and potassium excretion were elevated in the lithium over sodium injected animals (although not significantly so).

In accord with the results of the latter experiments are the results of the chronic balance experiment performed with pair fed rats. In this experiment, the first collection ended 2½ hours after lithium was injected into the experimental rats in order to make conditions comparable to the rat short acid excretion model used above. In this experiment, control rats received no sodium injection and there was no NH_4Cl intubation, however. Results for sodium, expressed as mean sodium excretion of the lithium injected animals minus mean socium excretion of the pair fed control, appears on figure 7, while potassium data appears in figure 8. It can be seen that in the first 2½ hours following the first lithium injection there is significant sodium and potassium loss in the experimental animals as compared with their controls. While experimental con-

ditions are not exactly similar to the balanced short acid excretion rat model studies, they support the findings in those experiments that lithium injection acutely causes increased sodium and potassium excretion.

The remainder of the experiment, designed to monitor sodium and potassium balance as lithium polyuria was induced, indicates similar excretion patterns for sodium and potassium during this period, with early net sodium and potassium retention (day 1 and 2) in experimental versus control groups, followed by impressive net loss of urinary sodium and potassium as polyuria becomes more profound (day 3-6). It may also be seen that, although notreflected in the first 2½ hour period (in accordance with the rat short acid excretion model), significant rises in urinary volume occur in the lithium group with the first 24 hour period.

II. Human Studies

A short acid excretion test was utilized to illustrate the effect of lithium on acid excretion in humans. Results of the urinary pH data for the pre- and post-lithium test appear in figure 9. It can be seen that there is no apparent rise in urinary pH over baseline values two hours after oral ingestion of 28 meq. Li^+/70kg. At this time, NH_4Cl at .1g./kg body weight, was given orally in an effort to induce metabolic acido-

sis. It can be seen that 1, 2, and 3 hours after ammonium chloride ingestion, when there was significant lowering of the serum bicarbonate, subjects in the post-lithium test exhibited a blunted urinary pH response to the acid load which was statistically significant when compared to the pre-lithium test. Titratable acid and ammonia excretory rates, however, did not indicate an impaired acid excretory rate at any time in the post-lithium test, suggesting an absence of inhibition of acid excretion at this level of lithium dosage.

Discussion

I. Rat Studies

A. Acid excretion studies

The objective of the rat experiments described above was to determine the effect of rapid parenteral administration of lithium chloride on the ability of the rat kidney to acidify the urine. Responses were compared to those of animals similarly injected with both potassium and sodium chloride. Net acidification of the urine was monitored through measurement of urinary pH and urinary bicarbonate, titratable acid, and ammonium excretion, all of which are presently thought to be dependent on active hydrogen ion secretion in both the proximal and distal nephron (81,100). Ammonium chloride loading was employed to maximize small differences in the groups' abilities to both lower urinary pH and excrete net acid (taken as titratable acid + ammonium - bicarbonate excretion). An acute (2½ hour) protocol was chosen to minimize side effects of chronic ion ingestion which may alter acid excretory ability (eg. impaired concentrating ability (24), reduced glomerular filtration rate (95), altered adrenal activity (73), or elevated renal glutaminase activity (93).).

It was clear that under the conditions imposed in the ex-

periments lithium chloride profoundly inhibited urinary acidification in relation to the other two ions. As all animals within an experiment were subjected to the same metabolic loads of acid, it may be inferred that differences among the groups with respect to plasma bicarbonate and total acid excretion were due to differences in hydrogen ion secretory rates within the tubule. At the end of the moderate acidosis experiments, the lithium group demonstrated the lowest and the potassium group the highest serum bicarbonate levels and total acid excretion, reflecting respective rates of renal hydrogen ion secretion; values for both parameters in the potassium-injected group were intermediate, probably reflecting an intermediate rate of hydrogen ion secretion (although plasma bicarbonate in this group may also have been lowered by extrarenal hydrogen ion shifts (43,126)). Similarly plasma bicarbonates of the lithium-injected group tended to be higher than the sodium group in the severe acidosis experiment (although statistical significance was not achieved), while total acid excretion was decidedly lower in the former group, again reflecting lower hydrogen ion secretory rates in the lithium animals.

In analyzing the results of the rat acid excretion studies an attempt was made to identify what known determinant of renal acid excretion lithium might be affecting. The following factors were considered: 1) carbonic anhydrase activity; 2) extra-

cellular volume expansion; 3) renal intracellular acid-base status; 4) intratubular electrochemical gradients; and 5) coupled luminal sodium-hydrogen ion exchange.

Diminishment of renal carbonic anhydrase actibity is a phenomenon known to cause decreased urinary acidification through enhancement of proximal and distal tubular disequilibrium pH's (80,102,137). An effect of lithium on this enzyme seems unlikely in light of the data demonstrating absence of such lithium induced effect on red cell carbonic anhydrase activity.

Extracellular volume expansion is another factor which exerts an inhibitory effect on acid excretion (70,71,98). In an effort to separate out a direct effect of lithium exclusive of any effects of extracellular volume, (a factor not controlled for in the studies of Orloff et al (91) or Berliner et al (71)) acid excretion in lithium treated animals was compared to that in sodium injected controls. As data from the hematocrit studies illustrate, lithium treated animals in the course of the acid ecretion studies are less volume expanded than sodium treated animals. Yet total acid excretion in the lithium treated group is significantly below the sodium treated group. It is suggested, therefore, that the acid inhibitory effect of lithium is mediated through factors other than volume expansion.

Intracellular pH is also felt to be of importance in de-

termining acid excretory ability, through a regulatory action on tubular hydrogen ion transport rates (80,81). Although it is presently little more than conjecture, it is conceivable that lithium, as an ion which is accumulated intracellularly, may replace a portion of the hydrogen ion pool, thereby causing intracellular alkalosis and secondary depression of hydrogen ion secretion.

Alteration of intratubular electrochemical gradients is a fourth means through which intratubular hydrogen ion secretory rates may be altered. Lithium is an ion which is probably not resorbed distally (see introduction) and it may, therefore, be concentrated in this location within the tubule, thereby increasing intratubular electropositivity. Such an alteration would increase the electrochemical gradient against which hydrogen ion must be secreted, and may underly the depressed hydrogen ion secretory rates seen after lithium administration.

Lastly, coupled luminal sodium-hydrogen ion exchange is also felt to be important in at least a nonspecific way in regulating hydrogen ion secretion (81,94,100). Competitive or non-competitive inhibition between lithium and sodium for such coupled hydrogen ion movement may act to depress hydrogen ion secretion. This mechanism seems particularly plausible in light of the possible common pathways of tubular transport of sodium and lithium (see introduction) and it is strengthened by

the data presented in the present experiments suggesting increased sodium excretion during periods of decreased urinary acidification.

It is also of interest to speculate about the location of lithium's action within the tubule. A plot of urinary pH versus serum bicarbonate compiled from both moderate and severe acidosis experiments (figure 10) reveals that at very low serum bicarbonate levels urinary pH in the lithium animals approaches the limits reached by the sodium controls. Such a pattern of decreasing urinary pH only at very low levels of plasma bicarbonate is also seen in proximal renal tubular acidosis in humans (86), where steep hydrogen ion gradients are generated only when a proximal tubular bicarbonate "leak" is minimized by decreasing the filtered bicarbonate load. The similarity in acid excretory patterns between the present studies and human proximal renal tubular acidosis allows the conjecture that the action of lithium in inhibiting acid excretion may reside, in part, within the proximal tubule. The data do not, however, exclude an additional distal tubular acidification defect.

In agreement with previous studies (9,38,75,90,101,103,143), the present experiments have also confirmed that administration of acute loads of potassium ion also inhibits urinary acidification (although notnearly to the extent of the lithium

ion). This phenomenon, originally felt to be due to competition between potassium and hydrogen for secretion at the distal tubule level (6,38,90), it presently felt by some to be due to a potassium-induced intracellular alkalosis of the renal tubular cell (100), with secondary depression of hydrogen ion secretion throughout the nephron. Recent experiments, however, in which volume expansion was carefully controlled for (12,72), have concluded that most of the inhibitory effect of intravenous potassium administration is due to this effect rather than a specific effect of potassium ion on hydrogen ion secretory mechanism. The present experiments, by demonstrating relative inhibition of acid excretion in potassium versus sodium-injected groups (which were volume expanded to the same degree), support the supposition that at least part of the inhibitory effect of potassium infusion on acid excretion is due to factors other than volume expansion, and thus lend additional support to the hypothesis that potassium-induced inhibition of acid excretion occurs at the renal cellular level.

B. Electrolyte excretion studies

Results of the chronic acid excretion studies suggest that lithium administration stimulates both sodium and potassium excretion in the 2½ hours after its administration.

Increased sodium excretion induced by lithium administration is also suggested in the 2½ hour controlled diet acid excretion studies, in which sodium excretion in the lithium-injected group was comparable to that in the sodium-injected group, in which sodium excretion was probably elevated by virtue of the sodium injection. That potassium excretion was not shown to be significantly elevated in lithium treated animals in these studies may have been related to increased distal sodium loads causing supranormal potassium secretion in the sodium treated group; in this regard the paired balance study, in which lithium-injected animals are compared to untreated controls, is probably more valid an estimate of the effect of parenteral lithium on potassium excretion. Previous studies documenting increased potassium excretion shortly after infusion of lithium chloride was begun also support this conclusion (3,7,35,132).

As was mentioned above, enhanced sodium excretion in the first 2½ hours after lithium administration may be related to depression of sodium-hydrogen ion exchange thought to be of importance in urinary acidification (94,100). Increased potassium excretion may arise from increased distal sodium-potassium exchange which results from increased distal loads of sodium.

This is not to imply that a direct reciprocal relationship between hydrogen ion and potassium ion secretion underlies this increased potassium excretion. Such a relationship has recently been shown not to exist (79). It is of interest to note that sodium and potassium wasting has also been noted in human poximal and distal renal tubular acidosis (44,86, 113), with similar mechanisms suggested to explain the phenomena.

In the chronic balance study, striking chronic increases in levels of both sodium and potassium excretion was noted shortly after an initial period of sodium and potassium retention (excluding the first 2½ hours of the study). This initial retentive pattern for sodium is quite similar to that described in previously in humans after oral lithium ingestion (3,63,87 132,133) (see introduction); potassium retention has, however, not been previously documented. Explanation for these initial retentive patterns are not readily apparent. The chronic daily sodium losses accompanying lithium administration have also been described in previous studies (99,106) and have been attributed to direct lithium toxicity at the tubular level. While this is a distinct possibility, both sodium and potassium loss may in addition be caused by chronically induced renal acidification defects (see above) which may attend chronic lithium administration.

That polyuria in the lithium animals increases strikingly

at a time when sodium and potassium wasting become profound could also suggest electrolyte loss secondary to the lithium-induced vasopressin-sensitive diabetes insipidus. Studies of electrolyte excretion patterns in both hereditary (51,135) and surgical (37) hypothalamic diabetes insipidus have, however, suggested only moderate potassium depletion with normal conservation of sodium. It would seen unlikely that these losses are due entirely to vasopressin-insensitive diabetes insipidus.

Contrawise, however, it may be postulated that the chronic losses of sodium and potassium contribute to lithium polyuria and the lithium induced concentrating defect. This interpretation is especially appealing in light of the well-known vasopressin-insensitive concentrating defect caused by potassium (61). However, in light of previous work demonstrating disrupted corticopapillary gradients in states of hypokalemic nephropathy (82), studies demonstrating intact corticopapillary gradients for sodium in lithium polyuric rats (34) makes this unlikely. However, existing studies do not conclusively rule out vasopressin-insensitivity at the collecting duct level as additional cause for hypokalemic concentrating defects (60), and, with regard to the present studies showing decided potassium loss as polyuria increases, hypokalemic nephropathy can, at present not be entirely excluded as contributing to lithium polyuria.

II. Human Studies

The human studies, although showing differences in urinary pH attained after ammonium chloride loading in the post lithium experiments, failed to demonstrate significant differences in ammonium or titratable acid excretory rates. However, it may be noted that serum lithium levels averaged only .87 meq./L. midway through the experiment, a level significantly lower than that attained in the rat experiments (approximately 2.5 meq./L. at 2½ hours). It is of additional note in that the demonstration of urinary pH elevation in the post lithium experiments without depressions in ammonium or titratable acid excretory rates may, in part, be due to the greater sensitivity of the former determinations. Thus, although an acid excretory defect was not conclusively demonstrated in humans, it is felt that one may still occur at higher levels of lithium intoxication.

Summary

Experiments were designed to detect acid excretory defects in rats and humans after acute administration of ionic lithium. 2½ hours after intraperitoneal injection of .15 M. lithium chloride (4 meq./kg. rat body weight), during which ongoing metabolic acidosis had been induced with oral ammonium chloride, lithium-injected rats demonstrated strikingly increased urinary pH and bicarbonate excretion, and decreased ammonium and titratable acid excretion, in comparison to both sodium and potassium-injected rats. Potassium-injected animals demonstrated a much less striking relative acidification defect in relation to sodium-injected animals. Human studies designed to detect a similar acid excretory defect failed to do so; serum lithium levels, however, were significantly lower than those in the rat studies.

Electrolyte excretion during the 2½ hours of the experiment revealed significantly increased excretion of sodium in the lithium treated as compared with the sodium treated controls. Studies designed specifically to detect lithium-induced electrolyte excretion abnormalities demonstrated increased excretion of both sodium and potassium in the 2½ hours following .15 M. lithium chloride injection (4 meq./kg.), when compared to pair fed controls receiving no ionic injection, and further

demonstrated striking losses of both sodium and potassium when daily injection were carried out over a six day period.

It was concluded that ionic lithium, at proper dosages, caused a marked retardation of acid excretion in rats at a time when sodium and potassium excretion is elevated. An inhibition of coupled luminal hydrogen-sodium ion exchange within the proximal tubule is the underlying mechanism suggested by the present data.

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Appendix A

Table 1

Effects of acute LiCl, NaCl, and KCl administration on arterial and urinary acid-base parameters during moderate metabolic acidosis

Group	V (ml./150min/kg)	Arterial Blood pH	Plasma HCO_3 (meq./L.)	U_{pH} at 150 min.	$U_{HCO_3^-}$* (meq./150min./kg)	T. A. (meq./150 min./kg.)	NH_2 (meq./150min./kg)	Total Acid Excretion (meq./150min./kg)	Plasma Li^+ (meq./L.)
LiCl injected rats (19)	5.07 ± .78	7.21 ± .02	14.44 ± .43	7.32 ± .12	.077 ± .030	.015 ± .003	.258 ± .024	.197 ± .041	2.49 ± .11
NaCl injected rats (15)	7.08 ± .94	7.29 ± .02	19.08 ± .46	5.80 ± .05	<.01	.070 ± .007	.637 ± .047	.726 ± .053	—
KCl injected rats (21)	18.83 ± 1.9	7.27 ± .02	15.39 ± .35	5.97 ± .07	<.01	.078 ± .031	.485 ± .178	.539 ± .119	—
LiCl vs. NaCl P	>.05	<.001	<.001	<.001		<.001	<.001	<.001	—
LiCl vs. KCl P	<.001	<.005	>.05	<.001		<.001	<.001	<.001	—
NaCl vs. KCl P	<.001	>.05	<.001	<.05		>.05	<.02	<.025	—

Values are means ± S.E. Figures in parentheses indicate number of rats.
* not measured in urine specimens whose pH values were below 6.25.

Table 2

Effects of acute LiCl and NaCl administration on arterial and urinary acid-base parameters during severe metabolic acidosis

Group	V (ml./150 min./kg.)	Arterial Blood pH	Plasma HCO_3 (meq./L.)	U_{pH} at 150 min.	$U_{HCO_3^-}$* (meq./150 min./kg.)	T. A. (meq./150 min/kg.)	NH_2 (meq./150 min./kg.)	Total Acid Excretion (meq./150 min./kg.)	Plasma Li^+ (meq./L.)
LiCl injected rats (13)	6.37 ±.78	7.00 ±.04	8.22 ±.95	6.16 ±.17	<.01	.036 ±.006	.414 ±.066	.450 ±.068	2.44 ±.37
NaCl injected rats (12)	8.63 ±1.50	7.08 ±.05	9.62 ±.84	5.64 ±.06	<.01	.117 ±.017	.952 ±.090	1.069 ±.098	—
LiCl vs. NaCl P	>.05	>.05	>.05	<.01		<.001	<.001	<.001	—

Values are means ± S.E. Figures in parentheses indicate number of rats.

*not measured in urine specimens whose pH values were below 6.25.

Table 3

Effect of acute LiCl and NaCl administration on electrolyte excretion during moderate acidosis

Group	Na^+ Output (meq./150 min./kg.)	K^+ Output (meq./150 min./kg.)
LiCl injected rats* (16)	0.427 ± .058	0.533 ± .043
NaCl injected rats* (10)	0.511 ± .088	0.571 ± .059
LiCl vs. NaCl P	>.05	>.05
LiCl injected rats† (9)	1.230 ± .110	0.985 ± .100
NaCl injected rats† (10)	0.890 ± .140	0.772 ± .100
LiCl vs. NaCl P	>.05	>.05

* uncontrolled dietary intake
† controlled dietary intake

Values are means ± S.E.
Figures in parentheses indicate number of rats.

Appendix B

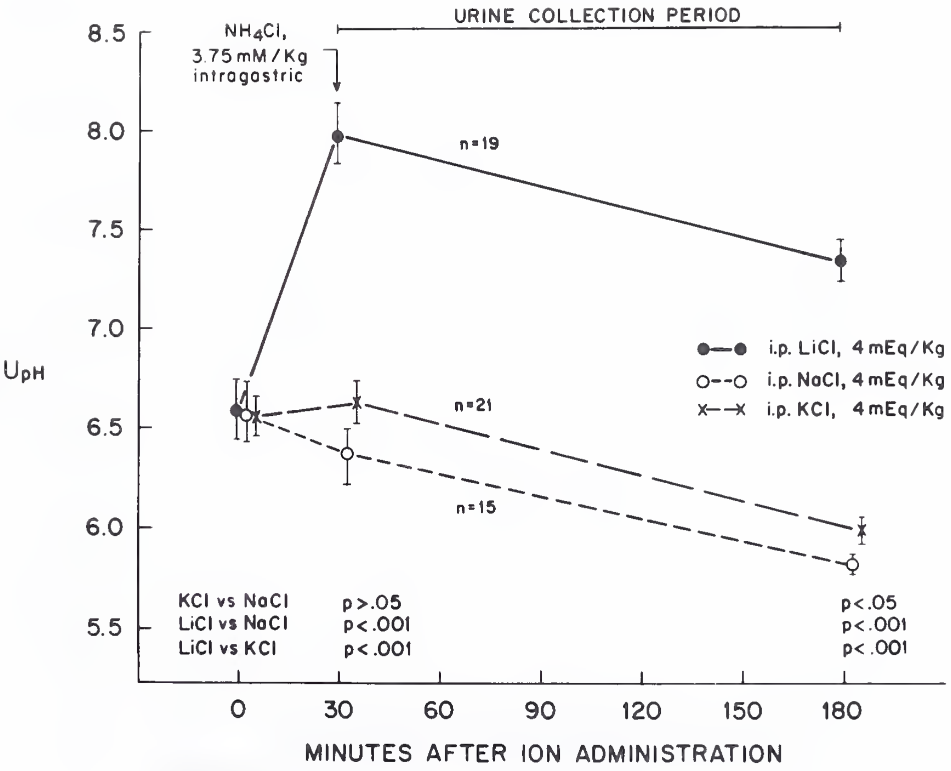

Figure 1. Time course and urinary pH data during induction of moderate acidosis with 3.75 mM intragastric NH_4Cl, in lithium, sodium, and potassium chloride injected animals (4 meq./kg.). Brackets indicate ± S. E. m equals the number of rats per group.

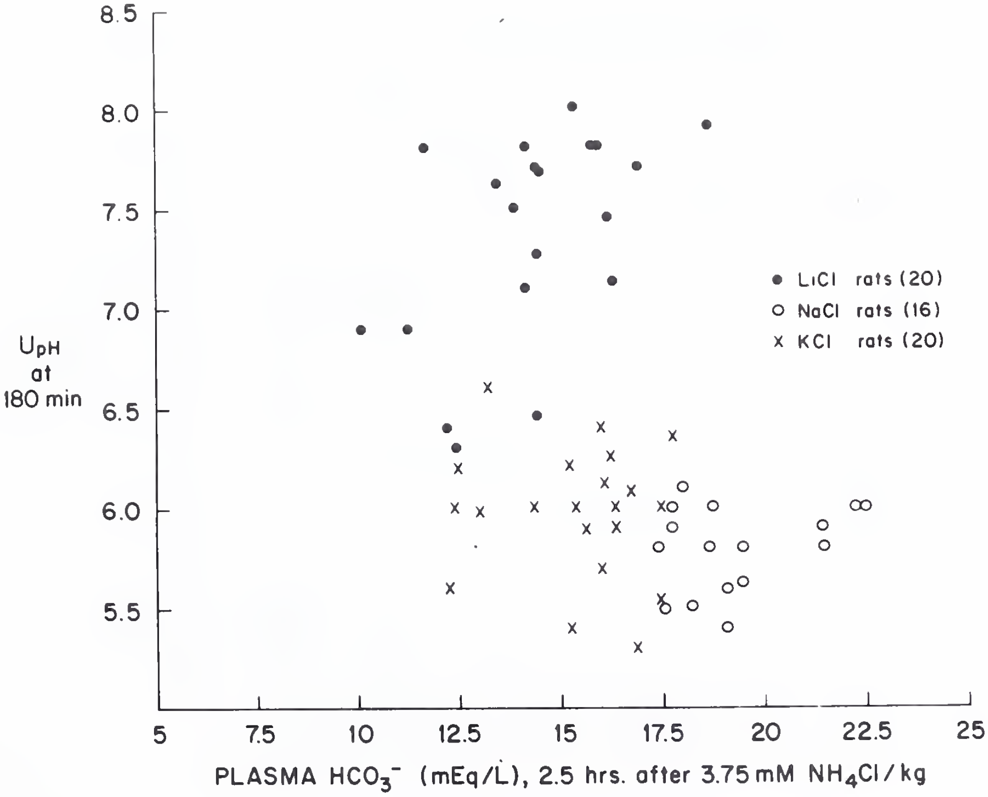

Figure 2. The effect of plasma bicarbonate alterations (induced with 3.75 mM./kg. intragastric NH_4Cl) on urinary pH in lithium, sodium, and potassium chloride injected animals (4 meq./kg.). Figures in parentheses indicate number of rats per group.

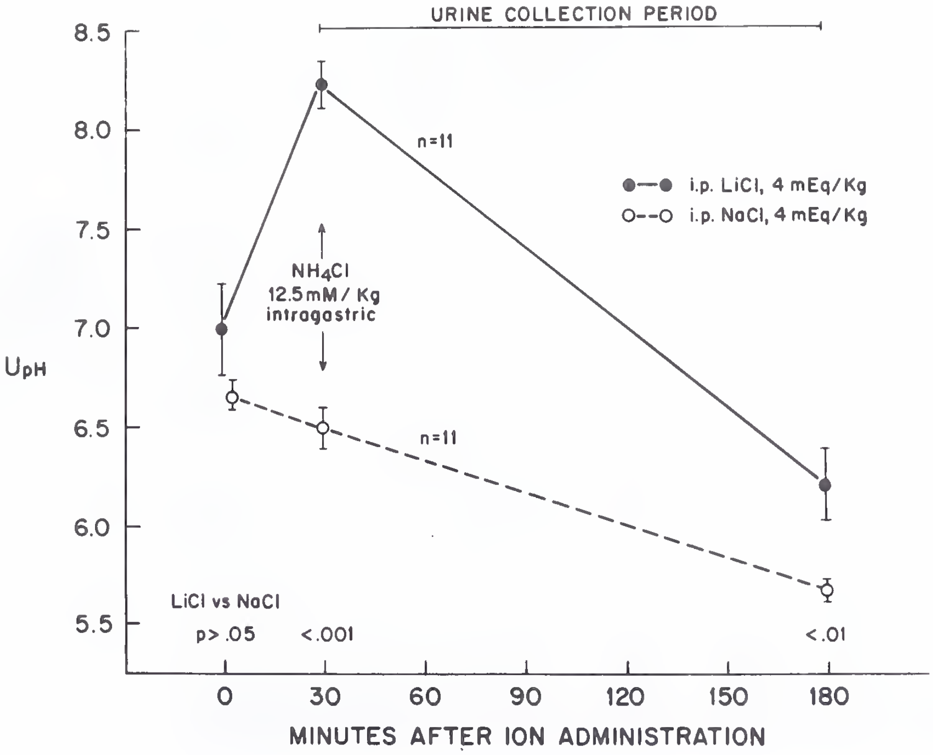

Figure 3. Time course and urinary pH data during induction of severe acidosis with 12.5 mM./kg. intragastric NH_4Cl in lithium and sodium injected animals (4 meq./kg.). Brackets indicate ± S. E. n equals number of rats per group.

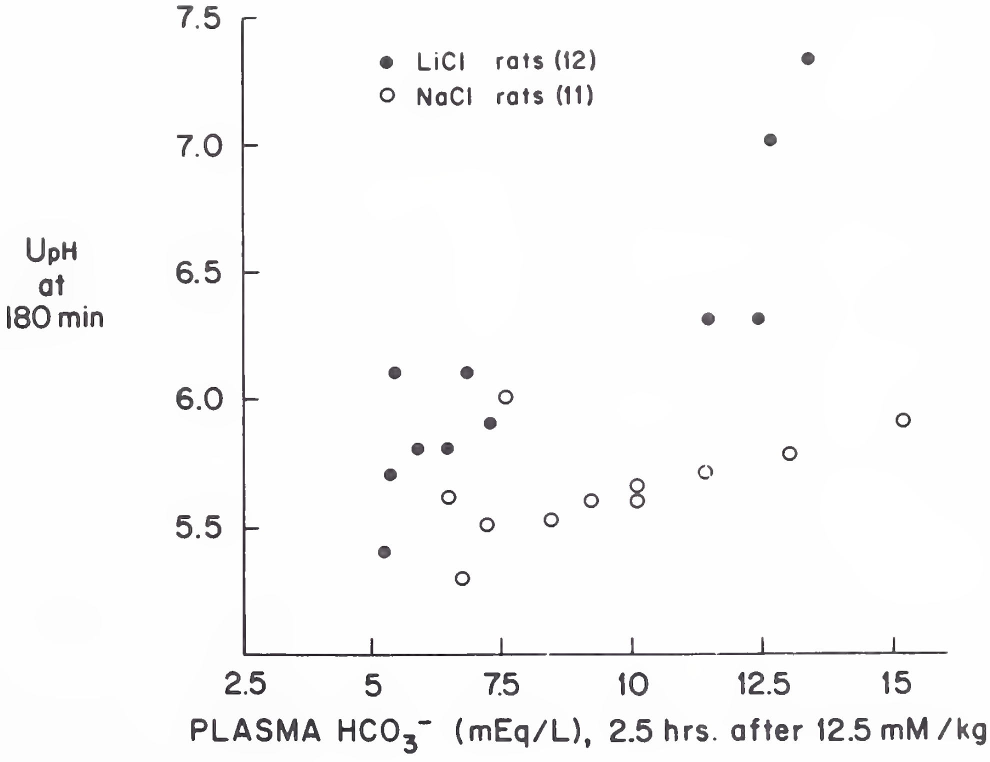

Figure 4. The effect of plasma bicarbonate alterations (induced with 12.5 mM./kg. intragastric NH_4Cl) on urinary pH in lithium and sodium chloride injected animals (4 meq./kg.). Figures in parentheses indicate number of rats per group.

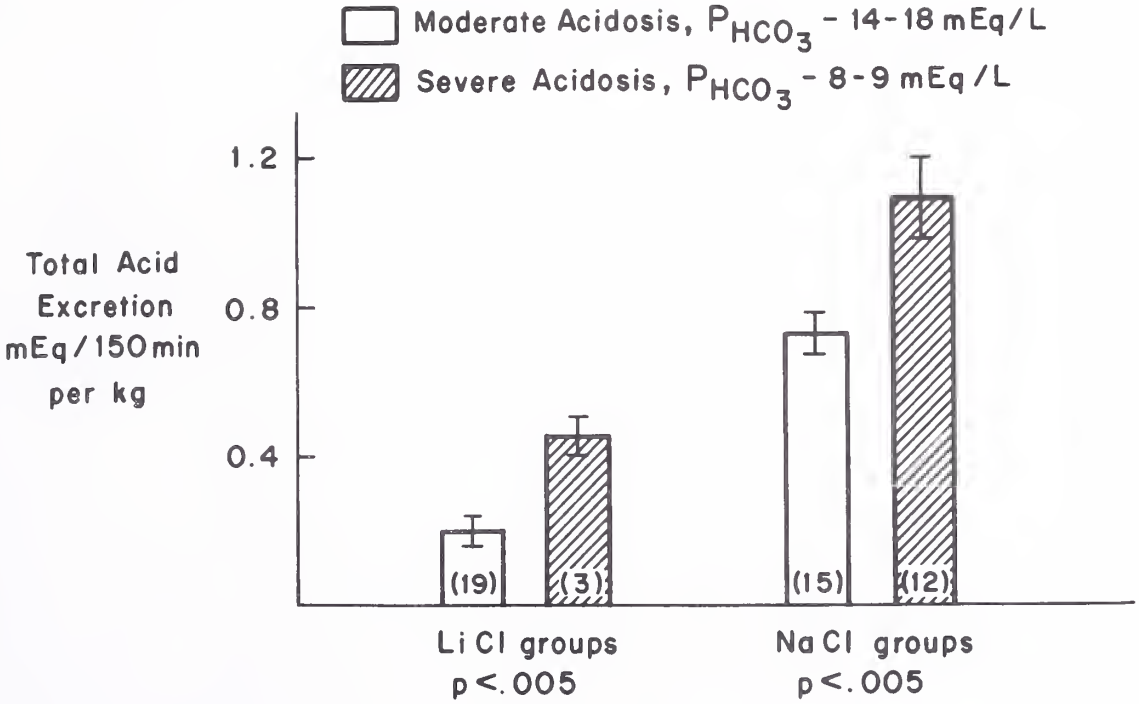

Figure 5. Acid excretory response of lithium and sodium chloride injected animals (4 meq./kg.) to moderate and severe metabolic acidosis (induced with 3.75 and 12.5 mM./kg. intragastric NH_4Cl, respectively). Brackets indicate ± S. E. Figures in parentheses indicate number of rats per group.

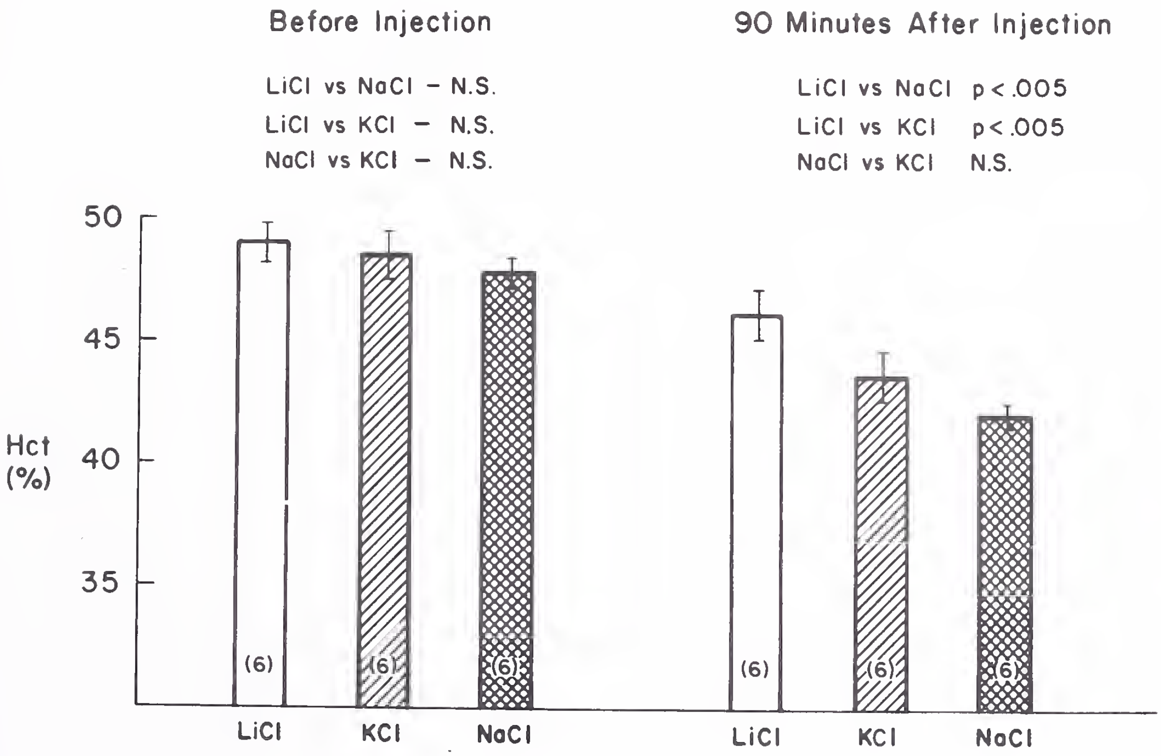

Figure 6. Comparison of hematocrits before and 90 minutes after injections of lithium, potassium, and sodium chloride (4 meq./kg.) (60 minutes after 3.75 mM./kg. intragastric NH_4Cl). Brackets indicate ± S. E. Figures in parentheses indicate number of rats per group.

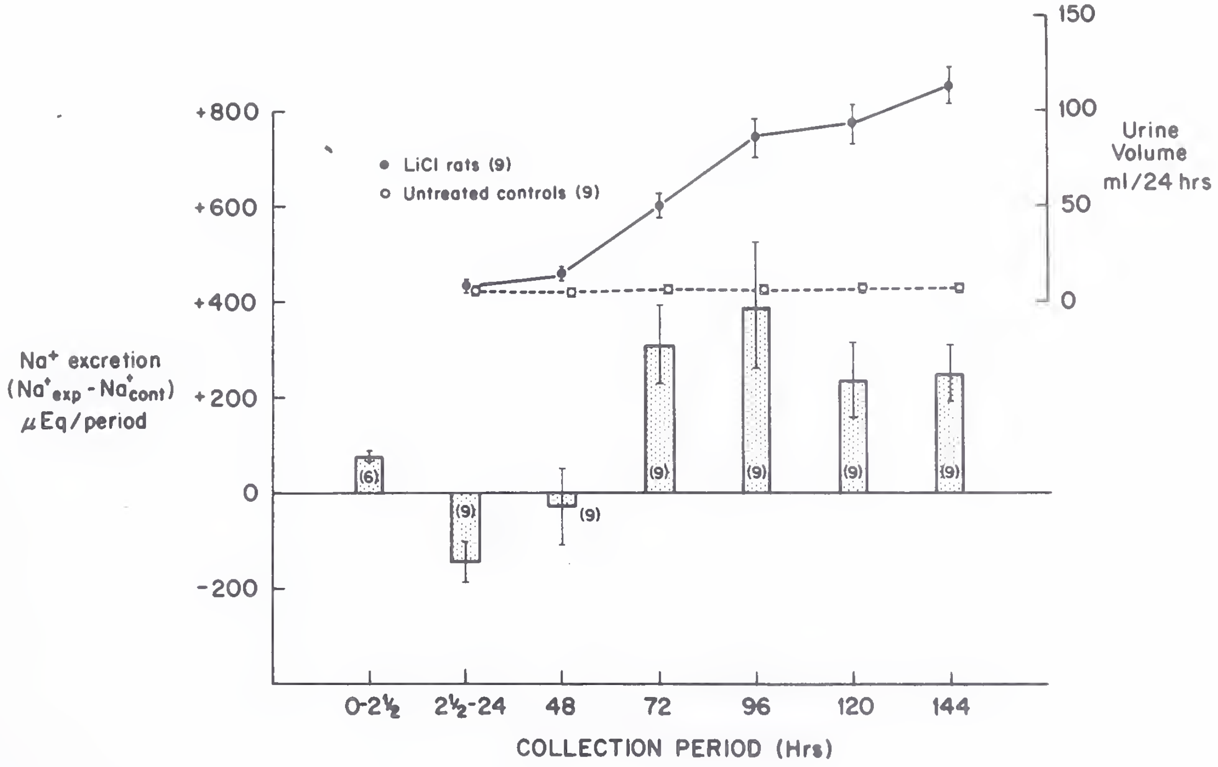

Figure 7. Comparison of urinary volumes and sodium excretion between animals receiving daily lithium chloride injections (4 meq./kg.) and uninjected controls. Brackets indicate ± S. E. Figures in parentheses indicate number of rats studied per day.

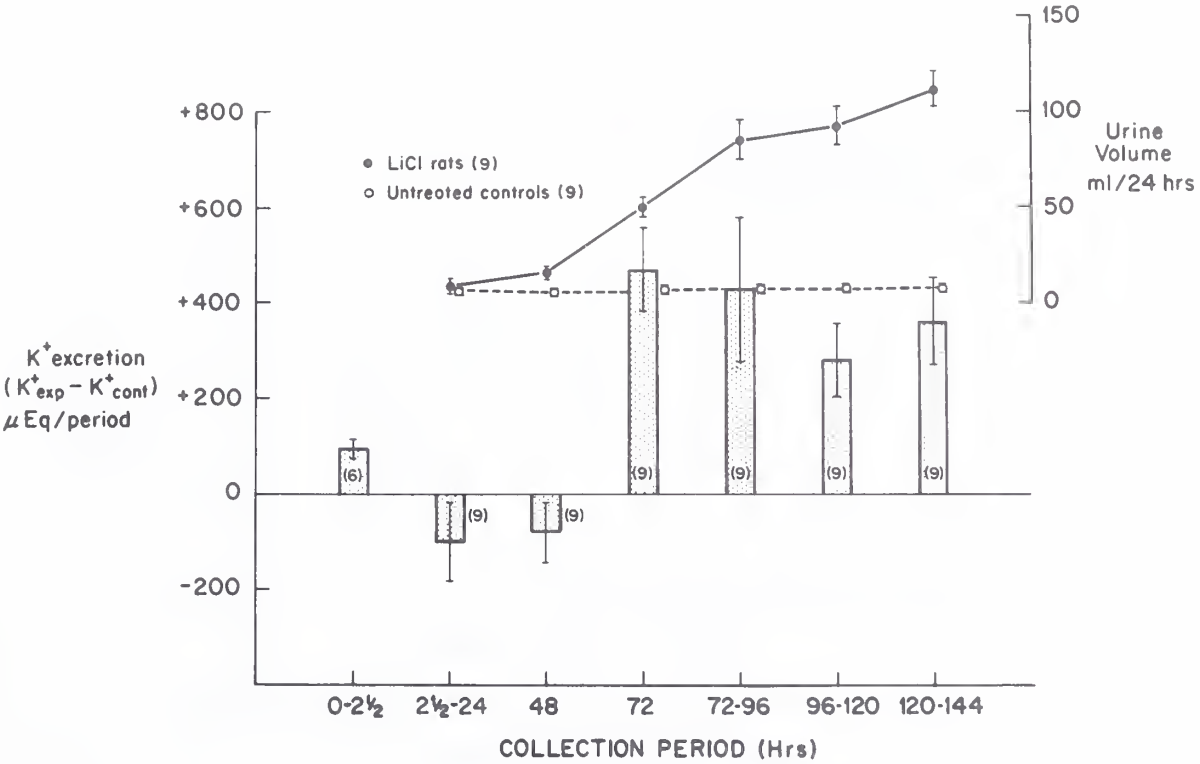

Figure 8. Comparison of urinary volumes and potassium excretion between animals receiving daily lithium chloride injections (4 meq./kg.) and uninjected controls. Brackets indicate ± S.E. Figures in parentheses indicate number of rats studied per day.

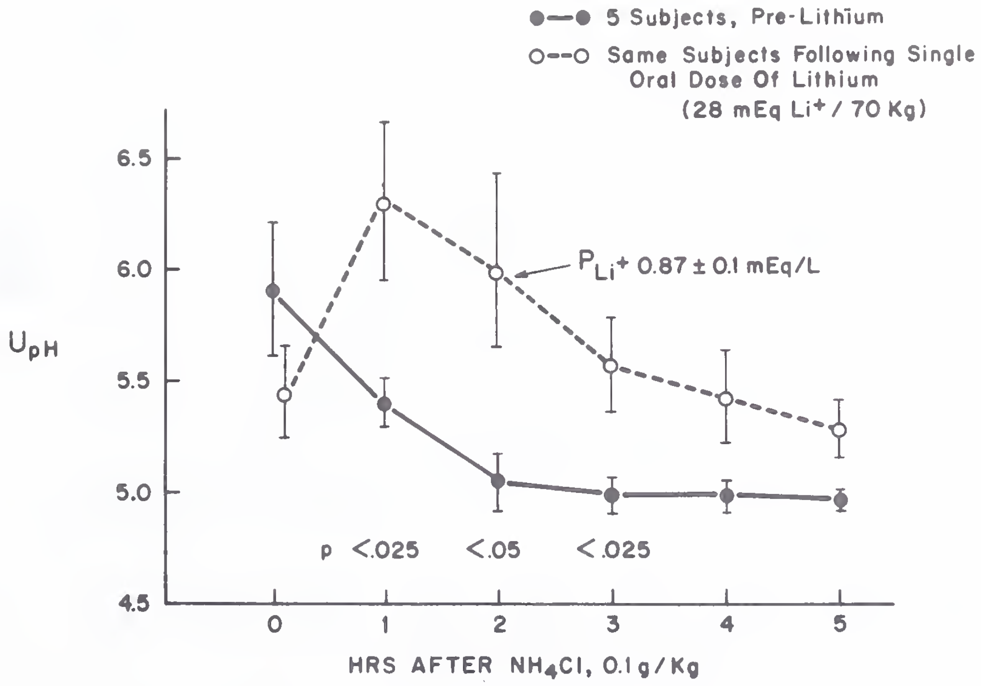

Figure 9. Urinary pH response in human subjects to p.o. NH_4Cl (.1 g./kg.) before and after ingestion of lithium (28 meq./70 kg.). Brackets indicate ± S. E.

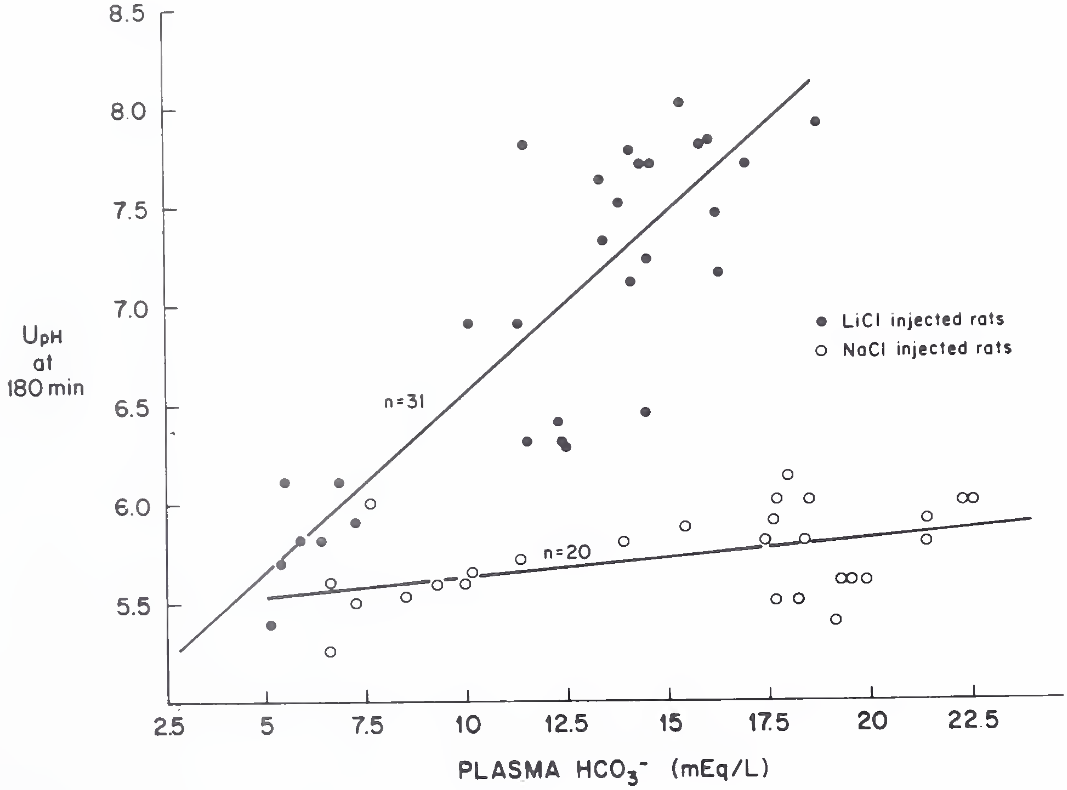

Figure 10. Correlation of urinary pH and plasma bicarbonate in lithium and sodium chloride injected rats (data compiled from moderate and severe acidosis experiments). n equals the number of rats per group. For the LiCl injected group $r=.8507$, $p<.001$; for the NaCl injected group $r=.4634$, $p<.02$.

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